

We claim:

1. A microfluidic apparatus for performing gel protein extractions, comprising:

a) an apparatus housing overlaid with an apparatus cover, wherein the housing has disposed therein a gel containing one or more proteins to be extracted and an electrolyte solution;

b) one or more fluidic channels containing an electrolyte solution, wherein the channels have a first end and a second end, and wherein the first end is disposed through the apparatus cover and is secured in position gel interface;

c) one or more outlet reservoirs having disposed therein an electrolyte solution, a first end of one or more outlet electrodes, and the second end of the one or more channels;

d) a high voltage power supply attached to a second end of the one or more outlet electrode for applying an electric field across the length of the on or more channels.

2. The apparatus of claim 1, wherein a ground electrode is connected to the apparatus housing;

3. The apparatus of claim 1, wherein each channel is connected to the high voltage power supply through an array of switches allowing one or more individual fluidic channels to be selected for extraction independently from the other fluidic channels.

4. The apparatus of claim 1, wherein the first end of each of the one or more fluidic channels terminates in a fluidic separation channel, comprising:

a) one or more fluidic extraction channels containing an electrolyte solution, wherein the channels have a first end and a second end, and wherein the first end is disposed through the apparatus cover and is secured in position at the gel interface;

b) one or more fluidic holding channels containing an electrolyte solution, wherein the channels have a first end and a second end, and wherein the first end terminates in the one or more fluidic extraction channels;

c) one or more outlet reservoirs having disposed therein an electrolyte solution, a first end of one or more outlet electrodes, and the second end of the one or more fluidic holding channels;

d) a high voltage power supply connecting to the one or more outlet electrodes through an array of switches allowing one or more individual fluidic channels to be selected for extraction independently from the other fluidic channels.

5. The apparatus of claim 1, wherein a detector is near the one or more outlet reservoirs for monitoring the extracted proteins.

6. The apparatus of claim 1, wherein the one or more fluidic channels are capillaries.

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7. The apparatus of claim 1, wherein the one or more fluidic channels are microscale channels

8. The apparatus of claim 6, wherein the capillaries are fused silica capillaries.

10 9. The apparatus of claim 1, wherein the fluidic channels are microfluidic channels formed in a planar glass substrate

10. The apparatus of claim 1, wherein the fluidic channels are microfluidic channels formed in a planar plastic substrate

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11. The apparatus of claim 1, wherein the fluidic channels are of a diameter which allows them to extract the one or more proteins in the gel in about two minutes or less.

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12. The apparatus of claim 1, wherein the fluidic channels are of a diameter which allows them to extract the one or more proteins in the gel in about ten minutes or less.

13. The apparatus of claim 1, wherein the outlet electrode is constructed of platinum.

14. The apparatus of claim 1, wherein the outlet electrode is constructed of gold.

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15. The apparatus of claim 1, wherein the outlet electrode is a thin film metal integrated into a glass substrate.

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16. The apparatus of claim 1, wherein the outlet electrode is a thin film metal integrated into a plastic substrate.

17. The apparatus of claim 6, wherein the capillaries have an outer diameter of between about 100  $\mu\text{m}$  and about 500  $\mu\text{m}$  and an inner diameter of between about 5  $\mu\text{m}$  and about 100  $\mu\text{m}$ .

18. The apparatus of claim 6, wherein the capillaries are between about 1 cm and about 50 cm long.

19. The apparatus of claim 1, wherein the electric field across the fluidic channel is between about 100 V/cm and about 1000 V/cm.

20. The apparatus of claim 1, wherein the apparatus cover and the apparatus housing create a gel chamber compartment that is pressurizable.

21. A method of transferring one or more proteins from a gel, comprising:

a) contacting a first end of one or more fluidic channels containing an electrolyte solution to one or more locations in the gel containing the one or more proteins, wherein

1) the gel is disposed within an apparatus housing overlaid with an apparatus cover and the housing has disposed therein an electrolyte solution and has attached thereto a ground electrode;

2) the one or more fluidic channels have a first end and a second end;

3) the first end of the one or more fluidic channels is disposed through the apparatus cover and is secured in position at or near the gel interface;

b) disposing the second end of the one or more fluidic channels in one or more outlet reservoirs, wherein

1) the outlet reservoir has disposed therein an electrolyte solution and a first end of an outlet electrode; and

2) a high voltage power supply is attached to the outlet electrode;

c) applying a high electric field along the length of the one or more channels, thereby extracting the one or more proteins from the gel and into the first end of the one or more channels;

d) concentrating the proteins near the first end of the one or more channels by electrophoretic stacking, and

e) transferring the proteins from the first end toward the second end of the fluidic channels.

22. The method of claim 21, wherein the one or more proteins are transferred from the first end toward the second end of the one or more fluidic channels in step (d) by a method further comprising:

a) stopping the high electric field across the one or more fluidic channels;

b) removing the first end of the one or more fluidic channels from the gel interface and transferring the first end into a reservoir of fresh electrolyte solution; and  
c) reapplying the high electric field to the one or more fluidic channels so that the one or more proteins are transferred from the first end toward the second end of the one or more fluidic channels for analysis and/or collection.

23. The method of claim 21, wherein the one or more proteins are transferred from the first end toward the second end of the one or more fluidic channels in step (d) by a method further comprising:

a) stopping the high electric field across the one or more fluidic channels;  
b) raising the one or more fluidic channels slightly above the gel interface; and  
c) pressurizing the compartment created by the apparatus housing and apparatus cover so that the one or more proteins are transferred from the first end toward the second end of the one or more fluidic channels for collection and/or analysis.

24. The method of claim 21, wherein the one or more proteins are transferred from the first end toward the second end of the one or more fluidic channels in step (d) by a method further comprising allowing the electric field to continually transfer the one or more proteins from the first to the second end while the first end of the fluidic channel is still contacted to the gel.

25. The method of claim 21, wherein the one or more proteins are transferred from the first end toward the second end of the one or more microscale fluidic channels in step (d) by a method further comprising:

a) stopping the high electric field across the one or more microscale fluidic channels;  
b) raising the one or more microscale fluidic channels slightly above the gel interface;  
and  
c) applying a negative pressure at the second end of the one or more fluidic channels relative to the compartment pressure within the apparatus housing so that the one or more proteins are transferred from the first end toward the second end of the one or more fluidic channels for collection and/or analysis.

26. The method of claim 21, wherein one or more instruments are located on or near the fluidic channels to analyze the one or more proteins.

27. The method of claim 26, wherein the instrument is a UV detector or a fluorescence detector.

28. The method of claim 21, wherein the one or more proteins are subject to digestion.

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29. The method of claim 28, wherein the one or more digested proteins are digested before transfer to the fluidic channel.

30. The method of claim 29, wherein the one or more digested proteins undergo in-gel digestion prior to transfer.

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31. The method of claim 29, wherein the digested proteins are fluidically transferred into a mass spectrometer from the first end of the microfluidic channel.

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32. The method of claim 29, wherein the digested proteins are fluidically transferred into a mass spectrometer from the second end of the microfluidic channel.

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33. The method of claim 29, wherein the digested proteins are fluidically transferred from the first end of the microfluidic channel onto a MALDI target plate for further analysis by a mass spectrometer.

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34. The method of claim 29, wherein the digested proteins are fluidically transferred from the second end of the microfluidic channel onto a MALDI target plate for further analysis by a mass spectrometer.

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35. The method of claim 29, wherein the proteolytic enzyme is trypsin.

36. The method of claim 28, wherein the one or more digested proteins are digested during transfer to the fluidic channel.

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37. The method of claim 36, wherein the one or more proteins undergo digestion in a membrane containing immobilized proteolytic enzymes positioned between the gel and first end of the one or more fluidic channels.

38. The method of claim 36, wherein the digested proteins are fluidically transferred into a mass spectrometer from the first end of the microfluidic channel.

39. The method of claim 36, wherein the digested proteins are fluidically transferred into a mass spectrometer from the second end of the microfluidic channel.

40. The method of claim 36, wherein the digested proteins are fluidically transferred from the first end of the microfluidic channel onto a MALDI target plate for further analysis by a mass spectrometer.

41. The method of claim 36, wherein the digested proteins are fluidically transferred from the second end of the microfluidic channel onto a MALDI target plate for further analysis by a mass spectrometer.

42. The method of claim 36, wherein the proteolytic enzyme is trypsin.

43. The method of claim 28, wherein the one or more digested proteins are digested after transfer to the fluidic channel.

44. The method of claim 43, wherein the one or more proteins is transferred directly from the one or more fluidic channels to a micro membrane reactor containing proteolytic enzymes for digestion.

45. The method of claim 43, wherein the one or more proteins is transferred directly from the one or more fluidic channels to a column reactor containing particles or beads immobilized with proteolytic enzymes for digestion.

46. The method of claim 43, wherein the one or more fluidic channels contains particles or beads immobilized with proteolytic enzymes for digestion.

47. The method of claim 43, wherein proteolytic enzymes for protein digestion are contained in solution within the fluidic channels.

48. The method of claim 43, wherein the digested proteins are fluidically transferred into a mass spectrometer from the first end of the microfluidic channel.

49. The method of claim 43, wherein the digested proteins are fluidically transferred into a mass spectrometer from the second end of the microfluidic channel.

50. The method of claim 43, wherein the digested proteins are fluidically transferred from the first end of the microfluidic channel onto a MALDI target plate for further analysis by a mass spectrometer.

51. The method of claim 43, wherein the digested proteins are fluidically transferred from the second end of the microfluidic channel onto a MALDI target plate for further analysis by a mass spectrometer.

52. The method of claim 43, wherein the proteolytic enzyme is trypsin.

53. The method of claim 21, wherein the one or more proteins are denatured in sodium dodecyl sulfate.

54. The method of claim 21, wherein the fluidic channels are capillaries.

55. The method of claim 21, wherein the fluidic channels are microfluidic channels formed in a planar glass substrate

56. The method of claim 21, wherein the fluidic channels are microfluidic channels formed in a planar plastic substrate

57. The method of claim 21, wherein the capillaries are coated with hydrophilic polymers such as polyacrylamide.

58. The method of claim 21, wherein a plurality of fluidic channels are arranged in an array, and the array contacts the gel.

59. The method of claim 21, wherein the electric field within each fluidic channel is individually addressable for extraction independently from the other fluidic channels.

60. The method of claim 21, wherein the plurality of fluidic channels may be positioned sequentially or simultaneously at various gel locations using a manual or automated positioning system, enabling individual or groups of fluidic channels within the array to sequentially or simultaneously extract multiple proteins from the various gel locations using a single extraction apparatus.

61. The method of claim 21, wherein the electrolyte solution contains Tris-HCl at a concentration of at most about 25 mM Tris-HCL at about pH 6.8.

62. The method of claim 21, wherein the gel is made of polyacrylamide or agarose.

63. The method of claim 21, wherein the gel is between about 1 mm and about 100  $\mu$ m thick.

64. The method of claim 21, wherein the gel is a gradient gel in the range of about 4% to about 20% polyacrylamide.

65. The method of claim 21, wherein the gel is a Tris/Tricine SDS polyacrylamide gel.

66. The method of claim 21, wherein the gel was used to perform 1D or 2D gel electrophoresis.

67. The method of claim 21, wherein the locations to place the one or more fluidic channels are visualized or imaged.

68. The method of claim 67, wherein the visualization is performed with Coomassie blue.

69. The method of claim 67, wherein the visualization is performed with silver staining

70. The method of claim 67, wherein the visualization is performed with SYPRO fluorescent dyes.